

Background: A normal, fully reconstituted immune system after hematopoietic cell transplantation (HCT) is critical for the control of post-transplant infections, establishment of graft tolerance and, in some cases, mediation of graft-versus-leukemia effects. Natural Killer (NK) cells, being the first in line of defense against tumors and infections, are also the earliest among lymphocyte populations to reconstitute and achieve functional maturity after transplantation. However, many HCT recipients with normal recovery of NK cells continue to suffer from complications including infections and disease relapse suggesting that different NK cell subsets may be responsible for anti-leukemic or anti-viral immune response. Here, we set out to determine in healthy individuals, whether different NK cell subsets (cytolytic or regulatory) elicit unique immune responses against different targets (leukemia cells or herpes viruses).

Methods: Peripheral Blood Mononuclear Cells (PBMCs) from 25 healthy donors were stimulated with different targets including a leukemic cell line (K562) and herpesviral (Epstein - Barr virus, EBV) infected cell lysate. A 5-colour flow cytometry based estimation of cytotoxicity (expression of CD107a, a surrogate marker for degranulation) and cytokine (IFN- γ) production was performed for both CD56^{bright}CD16^{neg} regulatory and CD56^{dim}CD16^{pos} cytolytic NK cell subsets.

Results: Different NK cell subsets were immunodominant against different targets. Leukemia (K562) – specific response includes both degranulation and IFN- γ production mediated by the cytolytic and regulatory NK cell subsets. On the contrary, EBV specific NK cell response was primarily characterized by degranulation and was dominated by cytolytic NK cells. A consistent shedding of CD16 was found associated with degranulation of cytolytic NK cells in response to EBV but not to K562 cells. Cytolytic NK cells in general exhibited a bifunctional immune response against both targets while regulatory NK cells were primarily IFN- γ producers.

Conclusions: NK cell subsets elicit a unique immune response against different targets (leukemia cells or herpesviruses). Assessment of posttransplant recovery of these target specific functional NK cell subsets will be more relevant for the prediction of transplant outcomes and may have future implications for the cellular therapy/prophylaxis of herpesviral disease or leukemia relapse.

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The Restorative Effect of Flt3-Ligand and IL-7 On CD4⁺ T Cell Homeostatic Proliferation During Graft-Versus-Host Disease

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Allogeneic hematopoietic stem cell transplantation (SCT) is the best treatment for numerous types of hematological malignancies. However, graft-versus-host disease (GVHD) is the major cause of morbidity and mortality and its effect on T cell regeneration greatly exacerbates the immunodeficiency normally associated with this treatment. As a result, patients with GVHD are profoundly lymphopenic and T cell reconstitution typically takes several months or years.

We previously reported that IL7Ra^{-/-} mice have a peripheral lymphoid niche that is highly permissive for homeostatic proliferation (HP) of CD4⁺ T cells. Given that CD4⁺ T cells regeneration is impaired during GVHD, we hypothesized that using IL7Ra^{-/-} bone marrow (BM) might improve their recovery in GVHD hosts. To study the impact of GVHD on the peripheral niche regulating CD4 HP, we used the

mouse model B6 into B6D2F₁. Since IL7Ra^{-/-} DCs support efficiently HP of naïve CD4⁺ T cells during lymphopenia, we used BM stem cells from B6IL7Ra^{-/-} mice and induced GVHD by adding 1x10⁶ B6 T cells. Finally, to understand the impact of GVHD on HP, we transferred CFSE labelled anti-HY CD4⁺ T cells (Marilyn) into GVHD hosts and measured their homeostatic proliferation 7 days later.

In non GVHD hosts, Marilyn T cells underwent robust HP while they completely failed in GVHD hosts. Absence of HP during GVHD was associated with a severe depletion of all DC subsets, including myeloid, lymphoid and pDCs. Low number of DCs during GVHD was in part due to their elimination by GVHD T cells but most importantly to a myelo-suppression affecting DC production from BM progenitor cells. Interestingly, treatment of GVHD mice with FLT3 ligand (FL) significantly increased the number of DCs, yet it was insufficient for restoring CD4 HP. Given that stromal cells and IL7 mRNA levels were also diminished in GVHD hosts, we provided both FL and IL-7 to GVHD mice and significantly restored HP of Marilyn CD4⁺ T cells in this setting.

Thus far, our data support a model wherein loss of CD4 HP during GVHD relates to lower numbers of DC and diminished systemic IL7.

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NK Cell Recovery and Costimulatory Molecule Profiles After Autologous Hematopoietic Cell Transplantation (HCT) in Multiple Myeloma (MM) Patients

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Background: Enhanced immune responses post autologous HCT is known to be beneficial for long term disease control in MM. Early responses are mediated by NK cells and alternate inhibitory/stimulatory pathways that include the costimulatory molecules. This pilot study assesses the expression of NK cytolytic receptor (CD16) as well as the stimulatory (OX-40, ICOS, 4-1BB, CD28, NKG2D) and inhibitory (PD-1 and CTLA-4) molecules on NK cells after auto-HCT in MM patients.

Methods: 22 patients with MM undergoing HCT, median age 59.6 years (36 - 71.4), were included in the study. Peripheral blood samples were taken 3 days prior to HCT and 14, 30, 60, 90, 180 days after HCT. At d180 post-HCT, 18/22 patients were receiving lenalidomide with d86 as median start date. NK cells and their costimulatory molecules were evaluated by flowcytometry using 2 six color panels of antibodies. One way ANOVA